

Assessment of Mycorrhizal Fungi Efficiency on Acacia's Growth Performance under Water Stress

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Abstract To assess mycorrhizal fungi efficiency on Acacia growth performance under water shortage condition, three leguminous plant species (*Acacia tortilis*, *Acacia ehrenbergiana* and *Acacia gerrardii*) were selected under greenhouse conditions in washed soil. The mycorrhizal fungal colonization was used to enhance plants growth under water deficit. Three watering levels; 85%, 75%, 50% and 25% of Field Capacity (FC) in the presence of Mycorrhizal and non-Mycorrhizal applied on grown trees for 5 months. This treatment impact on the plants was assessed by comparing plants heights, number of leaves shoot, root fresh, dry weight and Relative Growth Rate (RGR), and by measuring mycorrhizal colonization percentage and intensities. The results indicated that Arbuscular Mycorrhizal Fungi (AMF) significantly increased colonization percentage irrespective of acacia species. The maximum of root colonization percentage obtained at 75 % FC. Greater mycelium infection was observed at *A. tortilis*, *A. gerrardii* and *A. ehrenbergiana* (88.1%, 87.4%, and 86.4% respectively) at FC 75%, while the mycelium infection decreased at FC 25% at all species. The maximum vesicles were found with *A. ehrenbergiana*, *A. gerrardii* and *A. tortilis* (85.3%, 73.2%, and 53.5% respectively) at 75% FC, while the highest infection of Arbuscular (33.6%) was recorded with *A. ehrenbergiana* under 75% FC. Colonization intensity % significantly affect *A. gerrardii* registered highest mycelium intensity (66.3%) amended with 75% FC. The greater vesicles infection (62.6%) recorded with *A. ehrenbergiana* at the same FC, while maximum Arbuscular density (35.7%) with *A. ehrenbergiana* under 75% FC. Irrespective of Acacia species mycorrhizal fungi significantly enhanced the trees growth (plant height, leaves, shoot and root fresh weight, shoot dry weight and RGR) at 75% FC.

Keywords: water stress, acacia, mycorrhizal, growth, inoculum

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1. Introduction

Lack of vegetation cover, land degradation and reduced of agricultural production and forests require alternatives to enhance sustainability of natural resources under recent challenges [1]. Among the several nutrients influencing plants development during growth establishment, the effects of water availability on plants growth and how these compromise plant performance prevail studied by [2,3]. Soil types contributes positive or negative for water movement and water deficit [4]. Water stress, this can be limiting medium of plant growth and penetration. Friable soil faster percolation can also cause, in salts concentrate in the soil [5]. Water shortage is basically caused by periods of droughts, where evaporation rate greater than rainfall precipitations, thus leading to the depletion of soil water content. The plant needs water to survive and

develop. It is absorbs mineral elements in to the roots from the soil [6].

Most physiological processes need and regulating by water content; seeds, stems, leaves, vegetable growth, and fruit, as well as biological processes and chemical reactions [7,8,9]. Water stress during early growth lead to increase plant death rate and diseases [10].

Microhizal fungi, meaning fungus – root infection microbe, is beneficial form of symbiosis association between specialized fungi and plant roots [11]. Root System Enhancement, Improved Nutrient Efficiency, and Increased Water Absorption and Utilization [12]. Its application maintained plants to alleviate drought in many ways and situations [13]. Fungi mitigate drought stress directly by increasing the absorption surface and indirectly by increasing the biosynthesis of metabolic products that act as a response to water stress [14]. AMF important type of mycorrhizal fungi support host plant to take more water from the soil under drought period conditions [12]. Its

alleviate water stress by increasing the water absorption, nutrients uptake, stimulating proline synthesis, sugar formation and leading to growth promotion. AMF support plants to avoiding drought, by improving the relative water content and leaf water potential in plants [15].

The mycorrhizal fungal colonization presence does not meaning the enhancement of plants development under water stress. When root density reaches a certain point, any more absorption surface increase does not increase overall absorption, because the root–fungus interface is just insignificantly different from the roots [10]. Under water shortage, essential nutrients to accommodate like phosphorus and nitrogen is negatively affected and impedes [16,36]. Presence of mycorrhizae under water stress was proved significantly increased both nitrogen and phosphorus absorption [17].

Acacia's species are legumes, supply soil with nitrogen, which is one of the limiting nutrients for plant growth in arid and semi –arid areas in Sudan and Saudi Arabia [18] species provide gum, wood, forage and a good habitat of honey bees. The genus Acacia is currently gaining popularity due to its drought resistance, ability to enrich soil through nitrogen fixation and usage as fodder as well as shade and live fencing.

In this study, three pioneering plant species were selected: *Acacia tortilis*, *Acacia ehrenbergiana* and *Acacia gerrardii*, to assist mycorrhizal fungi efficiency on plant growth in poor soil under water shortage.

2. Material and Methods

2.1. Collection of Trees Seeds and Germination Test

Seeds of *Acacia tortilis*, *Acacia ehrenbergiana* and *Acacia gerrardii* were brought from Plant Production Department, Faculty of Foods and Agriculture sciences, King Saud University. The germination test of all species indicated that 80 – 90 % of seeds are viable. Seeds of Acacia species were boiled in water as a pre-treatment to overcome the hard coat and allow water imbibitions.

2.2. AMF Inoculum Preparation and Irrigation Levels

The mycorrhizal inoculum was produced using *Sesbania*, Onion, Maize and Sorghum with AMF were separated from the plants and produced root fragments approx. 1 cm. Sheared root inoculum carefully cleaned for the soil, stones and residues using distilled water and root surfaces were sterilized by ethanol alcohol. Roots fragments containing root inter-radical vesicles, arbuscules and mycelia. Roots were scattered and sprinkled on a flat vase and air dried at room temperature for 72 h. 5 g from roots fragments per 5 kg of sterilized soil 50% soil/50% sand (v/v). Root fragments were placed in the root zones of the growth at the green house under varying temperature between 21– 37°C. Pots were watered when depletion of soil water in pots reached 85%, 75%, 50% and 25% of field capacity

2.3. Growth Measurements

Treatments started after one month from sowing date. Plant heights and leaves per plant were taken every month till 5 months from treatment. In the final of experiment dry biomass (root / shoot) dry weight ratio (R/S) and (RGR). RGR of plant height estimated according to Hunt and Cornelissen, [34] formula as follows: $RGR(H) = (\ln H_2 - \ln H_1) / (t_2 - t_1)$, using Ln is natural logarithm, H1 and H2 as plant height (cm), and $t_2 - t_1$ as a time periods (month) on the last and first sampling date respectively.

After 5 months, the whole plants were removed carefully (with root system) and placed in paperback after gentle removal of soil from the root system to avoid detachment of finer roots. Directly transported to the laboratory for the following measurements per plant: shoots and roots fresh and dry weights and mycorrhizal colonization status.

2.4. Experimental Design

A greenhouse experiment was conducted as factorial experiment in Complete Randomized Design (CRD) with three replicates. Four seeds for each *Acacia tortilis*, *Acacia ehrenbergiana* and *Acacia gerrardii* were placed in 25 × 25 cm pots filled with 5 Kg sterilized sand soil. Watered under 85%, 75%, 50% and 25% of field capacity. Three species of *Acacia tortilis*, *Acacia ehrenbergiana* and *Acacia gerrardii* were randomly distributed and assigned for water stress treatment and inoculated with AMF inoculums and uninoculated.

2.5. Statistical Analysis

Data of this study were analyzed as factorial experiment in Complete Randomized Design (CRD) using Statistix 8 programme. Means were separated by using Least Significant Differences (LSD) and Tukey's test at ($p \leq 0.05$).

3. Results and Discussion

3.1. AMF Root Colonization Percentage

Figure 1 and plate 1 show the mean values of the mycorrhizal colonization ratios in the trees growing under different water regime levels. The percentage infection in the roots of different species with the mycorrhizal fungi varied significantly ($P \leq 0.05$), wide and independent variation was recorded irrespective of acacia species. Maximum mycelium infection was showed at *A. tortilis*, *A. gerrardii* and *A. ehrenbergiana* (88.1%, 87.4%, and 86.4%) at FC 75% respectively. While the mycelium infection decreased at FC 25% at all species. Vesicles structure percent not so far from mycelium. The greater vesicles were found with *A. ehrenbergiana*, *A. gerrardii* and *A. tortilis* (85.3%, 73.2%, and 53.5%) at 75% FC respectively. In the case of total infection with Arbuscular, the highest percentage of infection was recorded with *A. ehrenbergiana* under 75%FC (33.6%) and the lowest infection was found with *A. tortilis* 50% FC.

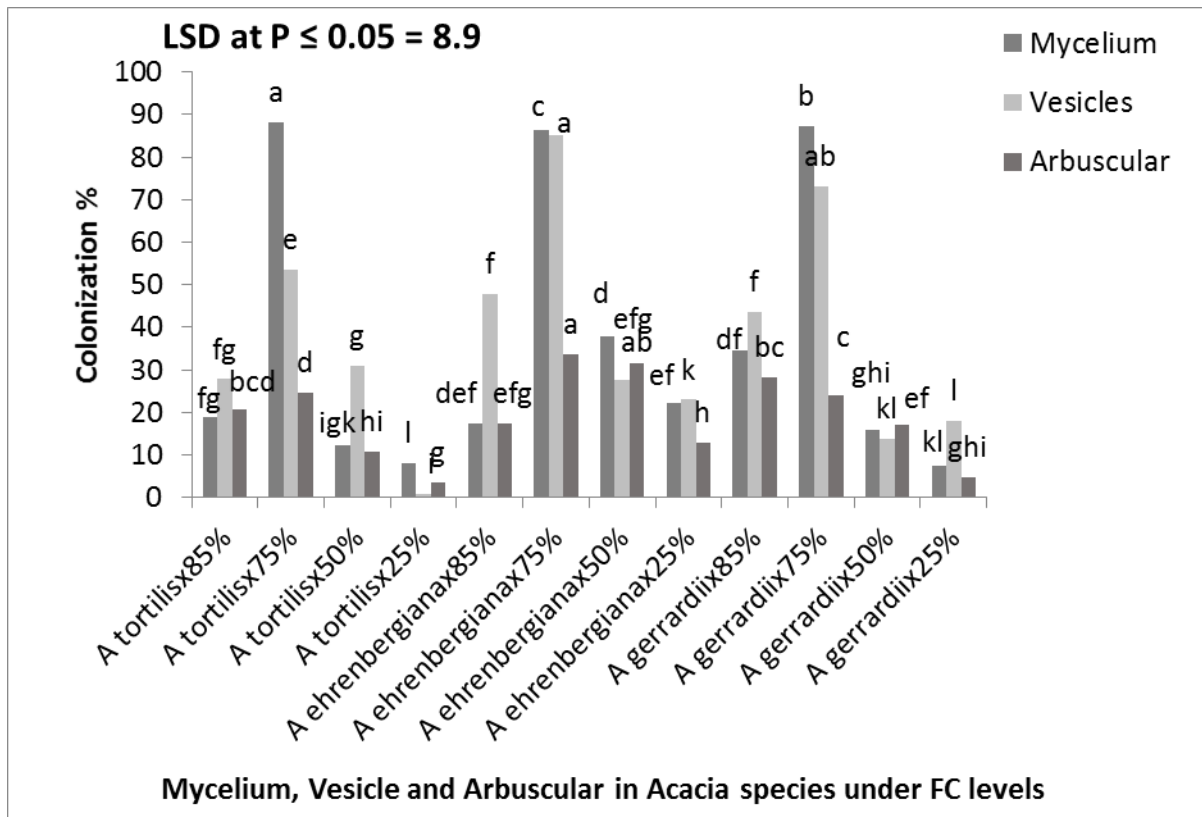


Figure 1. Interactions among species, inoculums and Field Capacity on roots colonization %

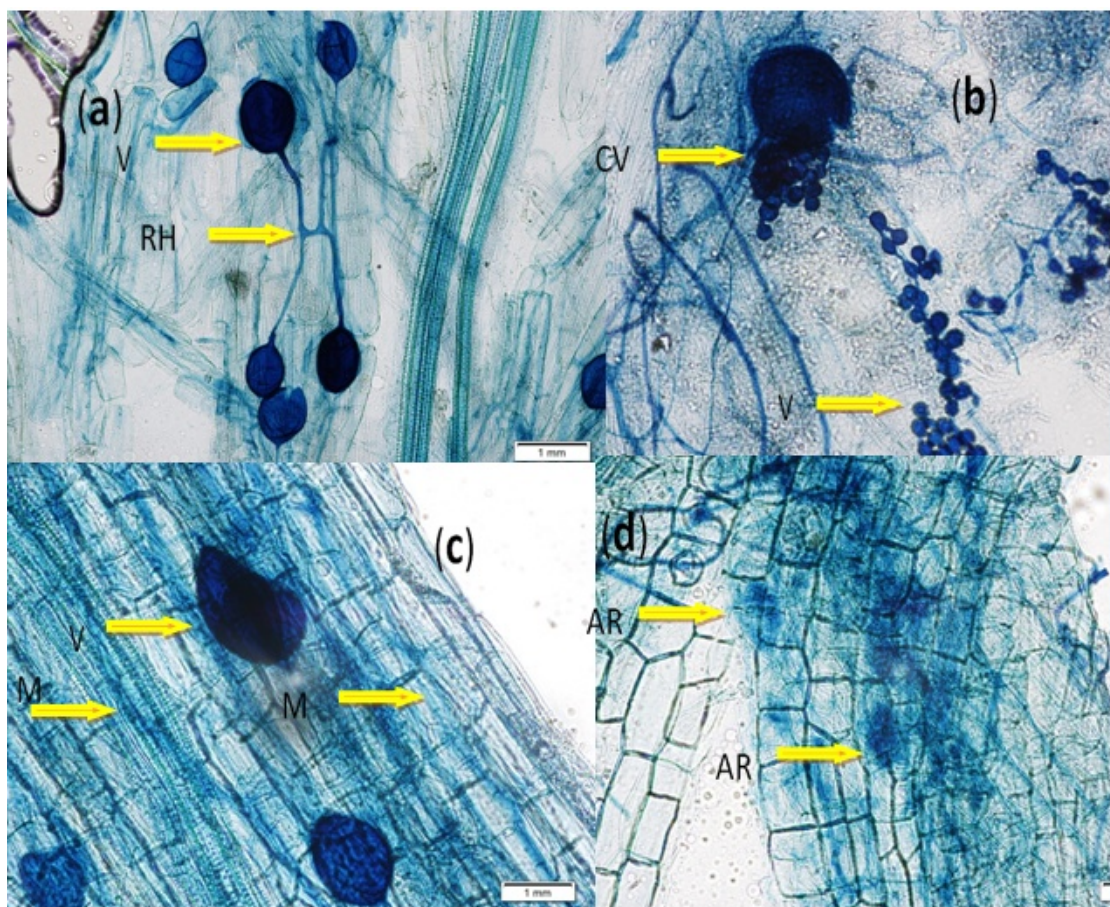


Plate 1. Photomicrographs of structural colonization of AMF in the roots (a, b & c) vesicles (V); running hyphae (RH) (b) crushed vesicles (CV); (c) mycelium (M) (d) Arbuscular (AR)

Table 1. Interactions among species, AMF and Field Capacity on roots Colonization Intensity %

Species	FC	Mycelium Means			Vesicles Means			Arbuscular Means		
<i>A. tortilis</i>		Mp	Mm	Ma	Vp	Vm	Va	Ap	Am	Aa
	85%	7.1	7.1	7.1	14.3	14.5	7.1	7.9	10.4	8.3
	75%	1.9	21.5	43.5*	8.3	28.7	43.8*	15.6	18.8	21.5
	50%	4.7	8.4	8.6	18.7	17.1	19.9	0.9	6	8.8
	25%	0.9	6.4	14.4	19.2	7.1	8.9	0.9	5.1	4.2
<i>A. ehrenbergiana</i>	85%	17.3	9.6	19.8	31.4	17.3	22.4	17.3	17.7	20.6
	75%	23.4	44.9*	54.7*	43.4*	50.4*	62.6**	26*	18.8	35.7*
	50%	17.3	20.3	0.9	25.3	25.4	18.6	24.1	7.6	3.9
	25%	11.5	16.7	4.3	12.7	11.1	18.7	13.7	19.4	2.2
<i>A. gerrardii</i>	85%	26.3	16.6	0.9	20.4	22.3	28.2	19.3	11.4	7.9
	75%	13.2	50.2*	66.3**	37.2	38.5*	48.4*	22	27.6	32.1*
	50%	12.8	7.6	14.1	18.7	25.2	13.8	4.4	3.2	3.2
	25%	0.9	0.9	8	5	7.7	6.6	0.9	0.8	0.7
LSD $P \leq 0.05$		Mycelium 13.24			Vesicle 11.46			Arbuscular 9.65		

The intensity of infection in individual tree species with mycelium along with coiled hyphae, vesicles, and Arbuscular was estimated as poor, moderate and abundant in each case. Infection varied significantly ($P \leq 0.05$) (Table 1) in each tree species under water deficit levels Table 1. Intensity of infection with mycelium, the maximum infection as poor, moderate and abundant types was recorded with *A. gerrardii* (66.3%) at 75%FC, followed by *A. ehrenbergiana* (54.7%) at the same FC level and the minimum was recorded with both of *A. tortilis* and *A. gerrardii* (0.9%) at 25%FC.

Intensity of infection with vesicles, the highest percentage was found with *A. ehrenbergiana* (62.6%, 50.4%) at 75%FC, followed by both of *A. gerrardii* and *A. tortilis* (48.4%, 43.8%) amended with 75% FC respectively, while *A. gerrardii* showed minimum intensity of infection. In contrast density percentages of infection by Arbuscular were very weak, highest percentage of abundant type was recorded with *A. ehrenbergiana* subjected to 75% FC (35.7%) and lowest density percentage found with *A. gerrardii* at 25% water deficit (0.7%). The mycorrhizal colonization for the selected acacia species under water deficit levels. Clearly mycorrhization decreased with the increase in FC our results are consistent with previous studies [4,19] and it is not so far from Ahmed *et al.*, [20]. Severity of the drought inhibit AMF performance but alleviated the negative effects of drought stress on the associated plant [15]. Certainly infection intensity percentage revealed the highest AMF colonization ratio, density varied significantly and independent variation was recorded in native AMF colonized numerous acacia species [18].

3.1.1. Spore Population Intensity

Spore population varied from 28 - 180/100 g in dry soils irrespective of acacia species variation depending on water regime. The highest spore population was recorded with *A. gerrardii* under 75% FC (180) followed by *A. tortilis* at 85% FC (175) and the minimum spores occurred at most of acacia amended with 25% FC. Undoubtedly AMF spores survive under water stressed condition. Our findings are consistent with Sarkar *et al.*, [21] who found *Glomus sp.*, *G. mosseae*, *G. fasciculatum*

and *G. aggregatum* in water stressed soil conditions and not so far from [20] AMF spores were tolerated severe drought conditions.

3.2. Growth Parameters

3.2.1. Effect of Water Stress on the Plant Height and Number of Leaves

Irrespective of Acacia species exposure of non-AMF inoculated plants to water stress resulted in a significant inhibition of growth as measured by morphological parameters at both plant heights after 2, 4 months and number of leaves / plant after 5 months. AMF significantly alleviated water deficit on plant height after 5 months ANOVA, (Figure 2a) illustrated plant height significantly ($P \geq 0.05$) higher at 75% FC and lower height observed at 50 % and 25% FC compared to uninoculated after five months, Among Acacia trees and water deficit levels *A. ehrenbergiana* showed better height (14.3 cm) subjected to 75% FC while the shorter height occurred at *A. tortilis* (3 cm) at 25% after 3 months from sowing date (Figure 2 b). Water stress and AMF had no significant differences between interactions of trees.

Table 2 demonstrates inoculation significantly ($P \geq 0.05$) increased number of leaves during plant growth; *A. tortilis* was recorded maximum leaves after 2 and 4 months (12, 23 leaves / plant) respectively amended with 75% FC and the minimum was observed with *A. ehrenbergiana* without AMF under 50% FC. Among species and water deficit levels; *A. gerrardii* registered more leaves number subjected to 75% FC. Increasing of water stress at 50% and 25% without treated positively decreased trees leaves number during severe water deficit time. Plant seedlings normal watering for one month let it establish well hence, AMF was added with regulated water deficit treatments. AMF needs some time to colonize, adapt and infect host plants as a results of statistically showed had no significant effect early months. Once root infected clearly shown in dual interaction after 3, 5 months comparison to non- inoculated similarly of Ndiaye *et al* [22] and Ahmed *et al* [20] mentioned that the AMF positively increased *A. senegal*, *A. seyal* heights.

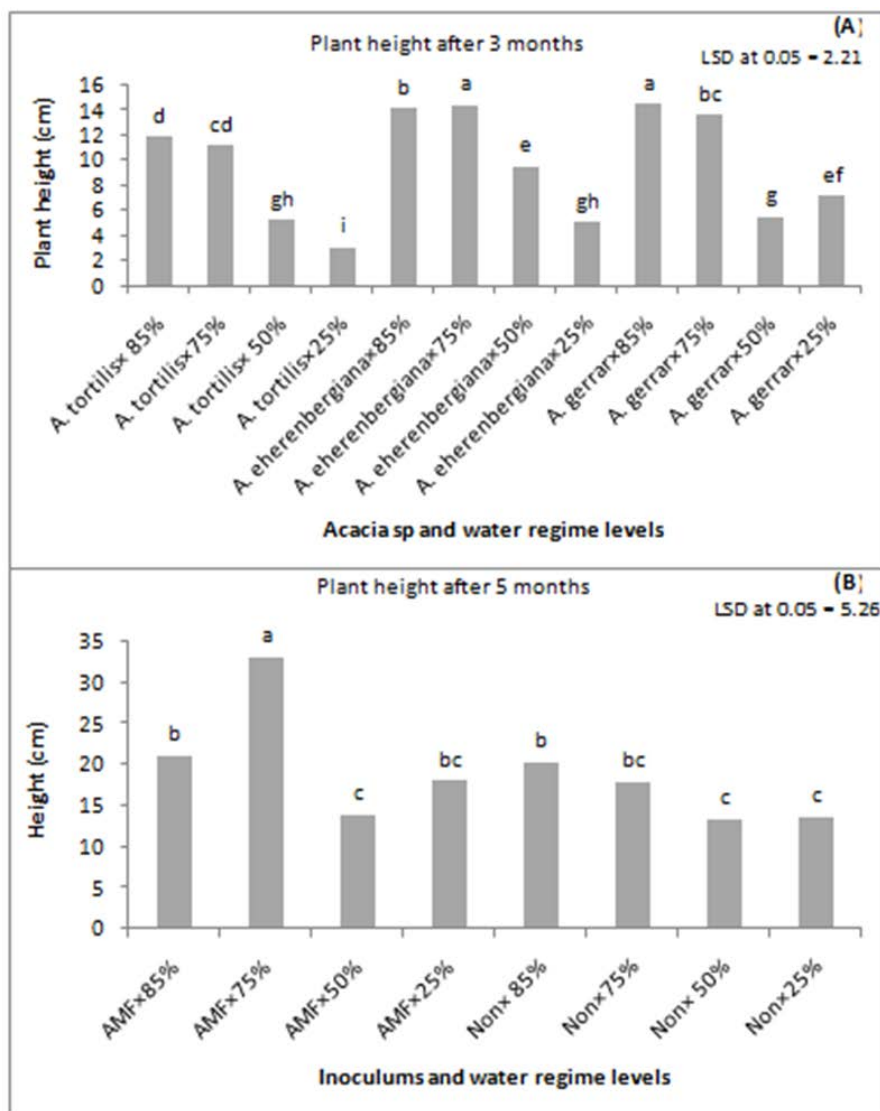


Figure 2. a and b: (a) Dual interaction among trees speices and water deficit levels on plant height after 3 months; (b) Dual interaction among inoculums and water deficit levels on plant height after 5 months

Table 2. Interactions among species, inoculums and Field Capacity on leaves / plant after 2 and 4 months from sowing date

Acacia species	Inoculums×FC%	Plant leaves after 2 months	Plant leaves after 4 months
A. tortilis	AMF×85%	6 defg	9 defg
A. tortilis	AMF×75%	12.3 a	23 a
A. tortilis	AMF×50%	7 bcde	11.3 defg
A. tortilis	AMF×25%	6 defg	11.3 def
A. tortilis	Non×85%	6.6 bcdef	10 defg
A. tortilis	Non ×75%	5.3 efg	8 fghij
A. tortilis	Non ×50%	6 defg	11.3 def
A. tortilis	Non ×25%	6.6 bcdef	12.3 cde
A. ehrenbergiana	AMF×85%	7 bcde	12.3 cde
A. ehrenbergiana	AMF×75%	9.3 b	17.3 b
A. ehrenbergiana	AMF×50%	9 bc	12.7 cd
A. ehrenbergiana	AMF×25%	6.7 bcdef	7.7 fghij
A. ehrenbergiana	Non×85%	4.7 efg	8.3efghi
A. ehrenbergiana	Non ×75%	5.3 efg	7 ghij
A. ehrenbergiana	Non ×50%	4.7 efg	4 j
A. ehrenbergiana	Non ×25%	5 efg	8 fghij
A. gerrardii	AMF×85%	6 defg	11 defg
A. gerrardii	AMF×75%	8.7 bcd	15.7 bc
A. gerrardii	AMF×50%	3.7 g	4.3 ij
A. gerrardii	AMF×25%	6.3 cdefg	8.7 defgh
A. gerrardii	Non×85%	6 defg	9.3 defg
A. gerrardii	Non ×75%	4 fg	4.7 hij
A. gerrardii	Non ×50%	6 defg	7ghij
A. gerrardii	Non ×25%	3.7 g	4.3 ij
LSD at 0.05		2.9	4.3

Mechanically plant in establish stage absorbing available nutrients from soil to build photosynthesis at leaves to growth increase. Exactly, positive shown at the followed month. Our results indicated a positive role of AMF inoculation to improve height, leaf number subjected to the four water deficit particularly, and non severe first two water deficits. This improvement of plant growth can be explained by the ability of AMF to help host plants absorb more water and nutrients from the soil by developing extra radical hyphae [23]. In the same line, of the Li *et al* [13] findings. The decline in cell growth leads to a reduction in organ size; hence, the first observable effect of water shortage on the plant can be seen in the limited size of leaves or plant height [24]. Due to nutrients consumption and reflected of increasing severe water deficit, trees leaves statistically had no significant effect after 20 weeks.

3.1.2. Effect of Water Stress on the RGR

Subjecting plants species to drought stress significantly ($P \geq 0.05$) increased trees RGR; *A. gerrardii* and *A. tortilis* amended with 75% FC recorded greater growth 32% and 31% respectively, and decreased at 50% and 25% FC; *A. ehrenbergiana* recorded minimum RGR (18%), overall growth rate observed greater at AMF-treated trees compared to uninoculated (Figure 3 a). Among inoculated AMF trees and without AMF showed passively significant differences ($P \geq 0.05$) trees subjected to 75%FC registered maximum RGR (34%) compared to AMF untreated amended with 25% FC (17%) (Figure 3b). ANOVA, illustrated had no significant differences in interaction between inoculums, FC% and acacia species.

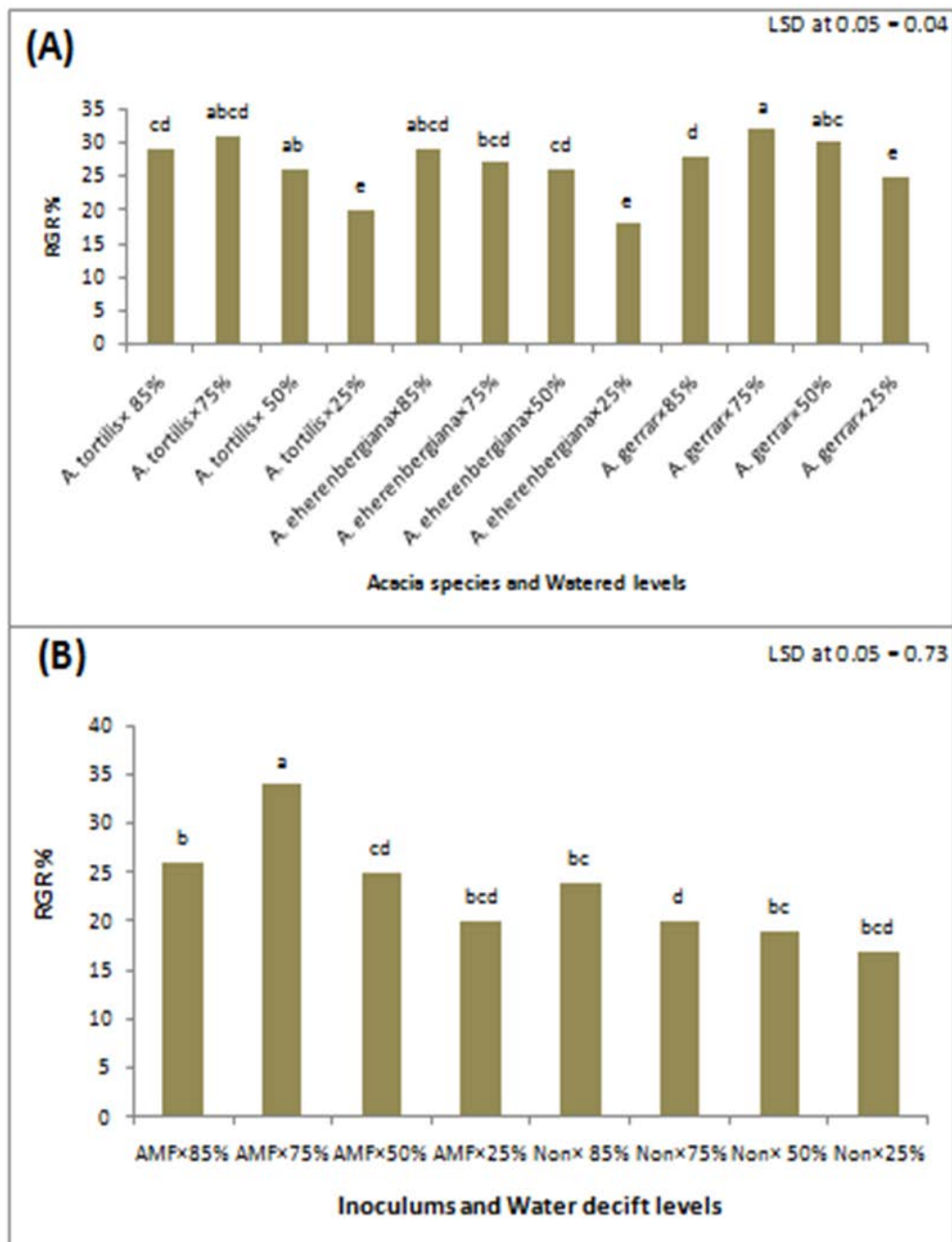


Figure 3. a and b: (a) Dual interaction among species and water deficit levels on RGR %; (b) Dual interaction among Inoculums and water deficit levels on RGR %

AMF inoculation particularly young seedlings had positive effects on plant growth performance [25] and contributed to the increase in the trees average RGR [26] it is not so far from Pavithra and Yapa [27] RGR was significantly increased with increased amount of water and decreased by reduced of water.

3.1.3. Effect of Water Stress on Shoot and Root Fresh Weight

Both shoot and root fresh weights were significantly ($P \geq 0.05$) improved by the presence of the mycorrhizal fungi particularly at mediated levels of water stress (75% FC). The shoot and root fresh weight of the mycorrhizal plants was (89%, 53%) and (78%, 62%) greater than the non-mycorrhizal trees amended with 75% FC at *A. gerrardii* and *A. tortilis* respectively. While the poorer shoot and roots vegetables amended with uninoculated *A. ehrenbergiana* and inoculated *A. tortilis* (13%), (6%) appropriately. The beneficial effect of the mycorrhizal was also observed in the absence of drought stress at 50% and 25% FC compared to uninoculated. (Table 3). Shoots dry weight indeed significantly was increased as explained in ANOVA (Table 3) *A. gerrardii* AMF – treated subjected to 85% and 75% FC maximum values (34%), (33%) respectively, while *A. ehrenbergiana* without AMF amended with 50% FC showed minimum value (5%). AMF inoculation did not have statistically interaction significant effect on Roots dry weight.

Regarding the shoots and RGR parameters the obtained results could be attributed to the fact that mycorrhizae symbiosis resulted in higher root distribution of the inoculated plants than in uninoculated [31]. This association may circumvent water regime either by

increasing the absorption of water and delivery [28]. Fungal hyphae exists on the seedlings' roots rhizosphere increase water and nutrient uptake, and this is directly reflected in an increase of shoot and root fresh and dry weights [29,30] and regulation of tolerance mechanisms, improving growth and yield under unstressed and stressed regimes [32]. AMF-treated young trees showed high shoot and root fresh weights. Untreated control trees subjected to severe water deficit showed the lowest shoot and root fresh and dry weights [20].

3.1.4. Effect of Water Stress on the Shoot and Root Dry Weights

Table 3 illustrated that AMF-treated trees showed the maximum average of shoot and root dry weight, whereas non-treated had lower shoot and root dry weight in all water deficit. Inoculated trees with highest fresh shoots and roots were recorded maximum shoots and root dry weight of *A. gerrardii* subjected to 85% and 75% FC and *A. tortilis* amended with 75% (34 %, 33%, 22%) respectively compared to untreated (Table 3). ANOVA, explained had no significant effect on root dry weight.

Considering the effect of water stress on reduction in a dry matter of plants, water scarcity decreases nutrients uptake, transfer, and consumption at each growth step leading to lower carbon storage and dry matter [35]. Inoculation with Arbuscular Mycorrhizae fungi had a significant effect on the increase in vegetative indicators of the plant under water deficit conditions. In this case, the dry weight of shoot and root were seen in inoculation process of trees AMF -treated while the lowest number of these traits was obtained for non-mycorrhizal treatments. Obtained results it is not so far from the findings of Sensoy *et al.* [33].

Table 3. Interactions among species, inoculums and Field Capacity on Shoots and roots fresh weight and Shoots and Roots dry weight

Species × FC%	Inoculums	Shoots Fresh Wt. %	Roots Fresh Wt.	Shoots Dry Wt.	Roots Dry Wt.
A.tortilis×85%	AMF	18 d	10 fgh	9 defg	25 a
A.tortilis×75%	AMF	78 a	62 a	22 b	19 ab
A.tortilis×50%	AMF	22 cd	7 h	8 efg	4 d
A.tortilis×25%	AMF	23 cd	6 h	8 efg	1d
A.tortilis×85%	Non	26 bcd	9 gh	11cdefg	5 c d
A.tortilis×75%	Non	24 cd	12 efgh	9 defg	6 b c d
A.tortilis×50%	Non	18 cd	10 fgh	8 efg	4 d
A.tortilis×25%	Non	22 cd	9 fgh	7 fg	2 d
A.ehrenbergiana×85%	AMF	42 bc	28 bc	17 bcdef	11 bcd
A.ehrenbergiana×75%	AMF	32 bcd	24 bcdef	18bcdefg	7 bcd
A.ehrenbergiana×50%	AMF	36 bcd	15cdefgh	17 bcde	7 bcd
A.ehrenbergiana×25%	AMF	34 bcd	15cdefgh	18 bcd	7 bcd
A.ehrenbergiana×85%	Non	28 bcd	15cdefgh	12bcdefg	6 d
A.ehrenbergiana×75%	Non	20 cd	22bcdefg	11 cdefg	8 bcd
A.ehrenbergiana×50%	Non	13 d	13defgh	5 g	7 bcd
A.ehrenbergiana×25%	Non	22 cd	22bcdefg	11cdefg	9 bcd
A.gerrardii ×85%	AMF	83 a	32 b	34 a	13 abcd
A.gerrardii ×75%	AMF	88 a	53a	33 a	19 abc
A.gerrardii ×50%	AMF	48 b	7 h	20 bc	3 d
A.gerrardii ×25%	AMF	13 d	26 bcd	8 efg	13 abcd
A.gerrardii ×85%	Non	37 bcd	25 bcde	13bcdefg	13 abcd
A.gerrardii ×75%	Non	23 cd	16cdefgh	10 defg	8 bcd
A.gerrardii ×50%	Non	30 bcd	23bcdef	11cdefg	11bcd
A.gerrardii ×25%	Non	23 cd	11efgh	10 defg	7 bcd
LSD at 0.05		0.23	0.13	0.07	0.13

4. Conclusion

The Current paper is concluded that AMF infection enhance Acacia growth under water stress. AMF inoculation amended with 75 % of Field Capacity alleviated water deficit and highest values of *Acacia tortilis*, *Acacia ehrenbergiana*, and *Acacia gerrardii*. Spore population varied widely and independently influenced by FC increasing particularly, at 25% FC. Irrespective of Acacia species mycorrhizal fungi significantly enhanced the trees growth at 75% FC.

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